Synchrotron X-ray Investigations of Mineral–Microbe–Metal Interactions

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Interactions between microbes and minerals can play an important role in metal transformations (i.e. changes to an element's valence state, coordination chemistry, or both), which can ultimately affect that element's mobility. Mineralogy affects microbial metabolism and ecology in a system; microbes, in turn, can affect the system's mineralogy. Increasingly, synchrotron-based X-ray experiments are in routine use for determining an element's valence state and coordination chemistry, as well as for examining the role of microbes in metal transformations.

KEYWORDS: X-ray, XAFS, X-ray microscopy, geomicrobiology, biogeochemistry

INTRODUCTION

More than 4000 mineral species have been identified, and the advent of molecular biology has revealed that Earth is populated by far more species of microbes than there are types of minerals (Skinner 1997). Archaea and Bacteria are the two prokaryotic domains of microscopic life that influence metal transformations in the environment (Woese 1987; Banfield and Nealson 1997). The most important properties of prokaryotes with regard to metal transformations are their size (typically approximately 0.5–5 microns) and the versatility of their metabolism. Prokaryotes can use a wide variety of electron donors. For respiration, they exploit many different electron acceptors besides O_2, including redox-reactive soluble ions and minerals. Often, the reactions involved in bacterial respiration result in the transfer of electrons (and thus the transformation of an element to a different valence state) or in alteration of an element's local chemical environment. All of these changes can have profound effects on the element's mobility through the lithosphere. Researchers are continually finding, in a remarkable array of environments, new microbial species capitalizing on chemical disequilibria in which enzymes catalyze otherwise slow reactions. Typically, cell densities of 10^6–10^9 cells per gram occur in soils and in the deeper subsurface (Barns and Nierzwicki-Bauer 1997). Their large cell densities, high surface-to-volume ratios, and versatile catalytic capabilities enable microbes to play major roles in metal transformations in subsurface and aquatic environments.

Many of the most pertinent questions regarding biogeochemical processes at microbe–mineral interfaces involve understanding which chemical reactions a microbe uses to gain energy, and the mechanisms by which it does this. The reactions often result in the transfer of electrons between living and nonliving entities. Synchrotron-based X-ray investigations of biogeochemical systems can identify the changes in an element's valence state and chemical speciation, which often result from microbially mediated electron transfer. These are powerful approaches for studying samples in their natural, often hydrated, states.

The double-headed arrows in FIGURE 1 represent some of the biogeochemical interactions near the mineral–microbe interface that are responsible for metal transformations. Extracellular organic material commonly found in soils and aquifers consists of a vast array of particulate and dissolved organic matter. These include humic substances, low-molecular-weight organic acids and carbohydrates, and a variety of microbially produced outer cellular polymeric substances (which include polysaccharides, DNA, RNA, and proteins) that may be attached to or exuded from the cell. These materials can interact with mineral surfaces and metals and can serve as a carbon source or electron shuttle for respiring microbes. Many prokaryotes can use soluble redox-active metal ions as electron acceptors for respiration, thus directly affecting the transformation of the metal. Biomineralization products (either associated with the mineral or bacterial surface or existing as a physically separate entity) can also affect metal transformations. Finally, in some instances, metal ions can be assimilated into the cell for use in metabolic processes. To date, the chemical interactions occurring at mineral–metal–microbe interfaces and the feedback-like responses between living and nonliving entities are poorly understood. However, such knowledge is imperative for addressing many issues related to global warming, carbon sequestration, and environmental cleanup, as well as most questions related to biogeochemical cycling of elements, to name just a few topics.

An ideal approach for investigating metal transformations at the mineral–microbe interface would employ a nondestructive, noninvasive method that could probe these

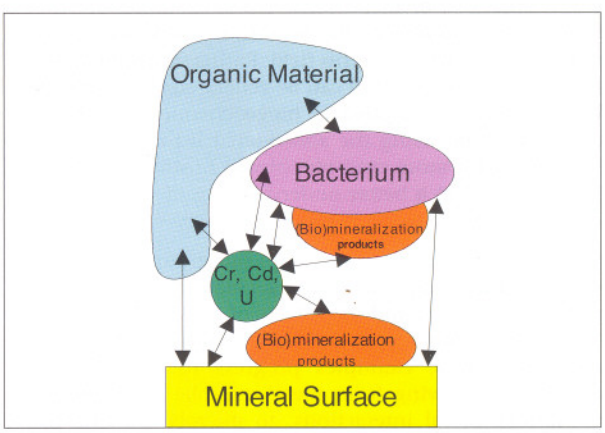

FIGURE 1 General types of chemical and physical interactions responsible for metal transformations at mineral–microbe–metal interfaces.

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transformations directly. Synchrotron-based X-ray absorption spectroscopy is such a method. In particular, the X-ray absorption fine-structure (XAFS) spectroscopy (Koningsberger and Prins 1988) techniques yield chemical and structural information for specific elements in a variety of unmodified—even hydrated—samples, including whole soils. The information provided by XAFS includes an element's oxidation state and coordination chemistry (in the form of the average number, distance, and atomic species of elements surrounding the element of interest).

Besides illustrating the complexity of mineral–microbe interactions and their role in metal transformations, FIGURE 1 also illustrates the spatial heterogeneities (less than a few microns) at the mineral–microbe interface. In some instances, to elucidate the interactions occurring at the mineral–microbe interface, the dimensions of the X-ray probe must be adjusted to place the vast majority of the X-rays in strategic positions relative to that interface. Recent advances in synchrotron-based X-ray imaging have led to the development of a class of techniques that address these requirements, where the electron beam of an energy-dispersive X-ray microanalysis experiment is replaced by an apertured or focused X-ray beam from a synchrotron source (Sutton et al. 1993; Kirz et al. 1995; Schulze and Bertsch 1995; Kemner et al. 2004). X-ray imaging microscopy is performed by focusing or aperturing the incident X-ray beam to a small spot and recording an image point by point by scanning the sample in the two dimensions perpendicular to the X-ray beam. At each point in the image, the energy-resolved X-ray fluorescence (XRF) spectrum is monitored. Selecting and plotting the intensity of the XRF of the element of interest yields an element-specific image of the sample. Currently, spatial resolutions of approximately 1 micron can be achieved with Kirkpatrick-Baez focusing optics (Eng et al. 1998). However, the use of high-resolution zone plates (Lai et al. 1992) has improved the spatial resolution of hard X-ray microimaging experiments to better than 100 nm. These techniques also can be combined with X-ray absorption spectroscopy techniques for "spectroscopic imaging," providing additional spatially resolved information in the form of an element's valence state and local chemical environment.

Numerous reviews have addressed the study of mineral–microbe interactions (i.e. geomicrobiology or biogeochemistry) (Beveridge 1988; Banfield and Nealson 1997; Fein 2000; Warren and Haack 2001) and the use of synchrotron radiation in environmental science (Schulze and Bertsch 1995; Brown et al. 2004). The present article briefly summarizes the use of synchrotron-based XAFS and X-ray microscopy by our group to investigate biogeochemical transformations of metal ions. These examples are by no means representative of the breadth of work performed by many other scientists in this field. Rather, they illustrate the types of information that can be generated by synchrotron-based techniques applied to biogeochemical systems. We recommend browsing the abbreviated list of references in this review for more examples of synchrotron-based experiments aimed at a better understanding of metal transformations at the mineral–microbe interface.

Below are three examples of ways our group has used synchrotron-based approaches to elucidate mineral–microbe–metal interactions and the effects of these interactions on the valence state and chemical speciation of contaminant elements. These examples progress toward increasing complexity, moving from metal–microbe interactions, to biomineral–metal interactions, to microbe–mineral–metal interactions.

XAFS INVESTIGATIONS OF INTERACTIONS BETWEEN CADMIUM–URANIUM AND THE MICROBIAL CELL WALL

Like previous studies of minerals, acid–base titrations and metal-uptake studies have shown that bacteria have reactive surfaces that can bind many metals (Beveridge and Murray 1980; Fein et al. 1997) and affect mass transport in aqueous systems. Developing models for contaminant transport in the environment necessitates detailed understanding of the contaminant's solution chemistry in a complex system of adsorbents. To address this need, surface complexation modeling approaches are currently being used to understand metal solution chemistry in the presence of bacteria (Koretsky 2000; Warren and Haack 2001).

Previous surface complexation modeling of the uptake of cadmium and uranium (a contaminant metal and radionuclide found at many contaminated sites) by *Bacillus subtilis* (a model microorganism found in many natural environments) biomass indicates that (1) at pH 1.5–3.0, uranium binds to protonated phosphoryl groups, but there is no cadmium uptake; (2) at pH 3.0–5.0, both uranium and cadmium bind to carboxyl groups; and (3) at higher pH, cadmium binds consecutively to deprotonated phosphoryl and hydroxyl sites (Fein et al. 1997; Fowle et al. 2000). Reactions are identified on the basis of the cell's elemental composition and the similarity of the deprotonation constants to those for aqueous acids.

To verify the surface complexation model and provide information for its refinement, we directly determined the average local atomic environment of uranium and cadmium bound to bacteria by making uranium L_3-edge and cadmium K-edge fluorescence XAFS measurements of wet, homogeneous *B. subtilis* biomass harvested at several pH values (Kelly et al. 2002; Boyanov et al. 2003). As in most of our work, we constructed theoretical XAFS models by using the program FEFF7 (Zabinski et al. 1995). Our theoretical models were based on the crystal structures of uranyl or cadmium acetate and phosphate. Structural XAFS parameters determined for the data are the number of carbon and phosphorus atoms surrounding the cadmium or uranium atoms, the distances between the cadmium and uranium and carbon and phosphorus atoms, and the relative mean square displacement about these average atomic distances. Additional details of the analysis of the XAFS data are provided elsewhere (Kelly et al. 2002; Boyanov et al. 2003).

FIGURE 2 shows the imaginary part of the Fourier transform of the cadmium K-edge XAFS data from solution standards, with a representative cadmium-biomass spectrum. The imaginary part of a Fourier transform of XAFS data can be thought of as illustrating a convoluted radial distribution function, where the location of the increased amplitude of the oscillation corresponds to the approximate radial distance between the atomic species that absorbs the X-ray and the atoms surrounding the absorbing species. The signals from carbon and phosphorus atoms are easily distinguished, as marked on the spectra. The carbon and phosphorus regions of the spectra for the pH-dependent cadmium-biomass spectra (not shown here) indicate a decrease in the phosphorus signal and an increase in the carbon signal with increasing pH.

The uranium XAFS results (Kelly et al. 2002) indicate that at extremely low pH (1.67), the uranyl ion (UO_2^{2+}) binds exclusively to phosphoryl functional groups, forming a monodentate inner-sphere complex[1]. With increasing pH (3.22 and 4.80), UO_2^{2+} binds increasingly to carboxyl

1 EDITORS' NOTE: terms such as functional group and inner-sphere complex are defined in the article by Sparks in this issue.

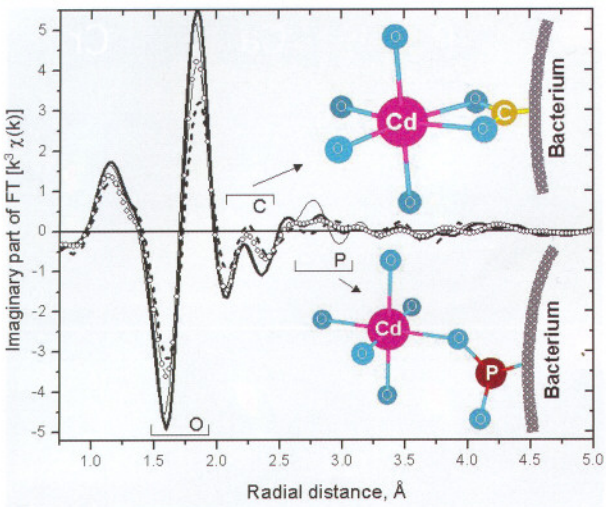

FIGURE 2 Imaginary part of Fourier-transformed XAFS data for Cd bound to the bacterial cell wall at pH 5.9 (circles). Data are compared to standards of hydrated (thick solid line), acetate-bound (broken line), and phosphate-bound (thin solid line) Cd in solution. The contributions to the XAFS spectrum from the C and P atoms in the corresponding ligand are noted.

functional groups, forming a bidentate inner-sphere complex. The cadmium XAFS results (Boyanov et al. 2003) indicate that at pH 3.0–5.0, phosphoryl groups are primarily responsible for the uptake of cadmium, while the additional sorption capacity at pH 5.0–7.5 is due to carboxyl binding. The onset of another binding mechanism observed at pH 7.8 was tentatively ascribed to further deprotonation of phosphoryl sites. The cadmium–phosphorus structural parameters are indicative of inner-sphere, monodentate binding to a singly deprotonated phosphoryl group, while cadmium–carbon structural parameters show inner-sphere, bidentate chelation in the cadmium–carboxyl complex. Results for simulations with the model developed to describe the chemical interactions between cadmium and the carboxyl and phosphoryl functional groups associated with the bacterial cell wall are shown as insets to FIGURE 2.

We identified the reactions responsible for cadmium and uranium uptake by *B. subtilis* by using XAFS to determine the local environment of the adsorbed uranium and cadmium. Our data in general corroborate results from surface complexation models; however, some details of the binding, such as ligand numbers, distances, and binding mode, can be observed only by XAFS. This information can be used in molecular calculations to independently constrain the stability constants used in surface complexation modeling. Taken together, bulk adsorption measurements, XAFS experiments, and *ab initio* calculations represent a powerful approach for determining and modeling metal speciation in metal–microbe–water systems.

XAFS INVESTIGATIONS OF URANIUM REDUCTION BY GREEN RUST

Although many microorganisms directly reduce a range of organic and inorganic contaminants, many elements are also reduced by reductants formed directly or indirectly by the metabolic processes of anaerobically respiring cells, particularly dissimilatory iron-reducing bacteria (DIRB) and sulfate-reducing bacteria. The DIRB are a diverse group of microorganisms that couple the oxidation of organic compounds or hydrogen to ferric ion (Fe^{3+}) reduction. The reduction of Fe^{3+} by DIRB typically results in the formation of a suite of ferrous (Fe^{2+}) species, including soluble Fe^{2+} complexes; Fe^{2+} surface complexes with organic and inorganic solid phases; and a host of Fe^{2+}-bearing minerals including magnetite, siderite, vivianite, and green rust (Lovley et al. 1987; Fredrickson et al. 1998; Ona-Nguema et al. 2002).

Green rusts are mixed ferrous and ferric hydroxides that have brucite-like layered structures consisting of alternating positively charged hydroxide layers and hydrated anion layers. Recent research showed that green rusts can reduce a number of organic and inorganic contaminants. These results suggest that green rusts may be highly reactive reductants in suboxic environments.

Besides demonstrating direct microbial reduction of U^{6+} by indigenous microorganisms isolated from sediments collected from an abandoned uranium mine (Suzuki et al. 2002), we recently reported the reduction of U^{6+} to U^{4+} by green rust (O'Loughlin et al. 2003). In both instances, the resulting solid phase was identified as nanoparticulate uraninite (UO_2). Analysis by uranium L_3-edge X-ray absorption near-edge spectroscopy (XANES) (Koningsberger and Prins 1988) of aqueous green rust suspensions spiked with uranyl (U^{6+}) showed that U^{6+} was stoichiometrically reduced to U^{4+} by green rust. Extended XAFS data for the U^{6+} reduced by green rust indicated the formation of a UO_2 phase. Fitting of data from the green rust samples to a theoretical model based on the crystal structure of UO_2 (see FIGURE 3) indicated that the number of nearest-neighbor uranium atoms decreased from 12 for the standard UO_2 structure to 5.4 for the uranium–green rust sample. With an assumed 4 near-neighbor uranium atoms per uranium atom on the surface of UO_2, the best-fit value for the average number of uranium atoms indicated the presence of UO_2 particles with an average diameter of 1.7 ± 0.6 nm (O'Loughlin et al. 2003). A schematic representing the uraninite nanoparticles identified in these experiments is shown as an inset to FIGURE 3.

The formation of biogenic green rusts by DIRB provides a means of coupling the redox cycling of iron to contaminant reduction in subsurface environments. Our results clearly indicate that U^{6+} (as soluble uranyl ion) is readily reduced by green rust to U^{4+} in the form of relatively insoluble UO_2 nanoparticles. This suggests that the presence of green rusts in the subsurface has significant effect on the mobility of uranium, particularly under iron-reducing conditions.

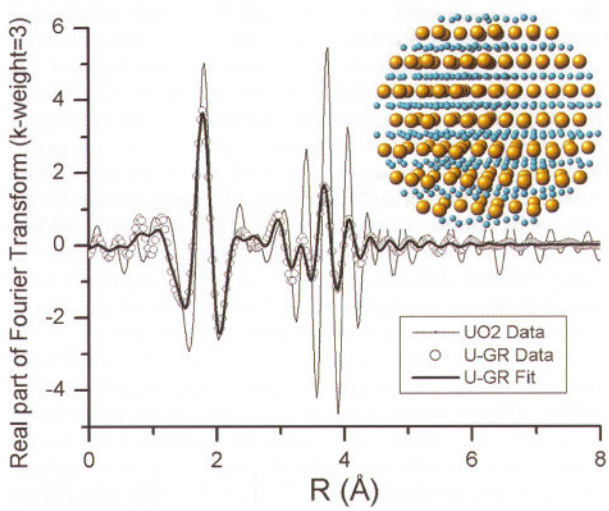

FIGURE 3 Real part of the Fourier transform of uranium L_3-edge XAFS data collected for a uraninite (UO_2) standard, a sample of green rust to which U^{6+} was added, and the fit to the green rust data. The data indicate the reduction of U^{6+} to U^{4+}, consistent with a UO_2 phase. Inspection of the data at 3–5 Å reveals a smaller uranium backscattering signal for the green rust sample than for the UO_2 standard, implying the formation of nanocrystalline uraninite. A model depicting the proposed uraninite nanoparticle is shown as an inset.

In addition, because these tiny particles can still be transported in an aqueous environment, precipitation of uranium as insoluble uraninite cannot be presumed to immobilize the metal. Thus, investigation of the transport of nanoparticles is currently a very important research area. Uranium XAFS studies of these systems can provide critical information concerning physical and chemical characteristics of these particles.

X-RAY MICROSCOPY ELEMENTAL AND REDOX ANALYSIS OF CHROMIUM INTERACTIONS WITH SINGLE BACTERIAL CELLS

Attachment of prokaryotic cells to surfaces often leads to major changes in metabolism and resistance to elevated concentrations of metals (Costerton et al. 1987). In addition, in many natural environments, bacteria exist predominantly in surface-adhered (biofilm) states rather than in free-floating (planktonic) states. Thus, understanding metal transformations at microbial interfaces and the role of microbes in transformations of metals in natural environments requires observation of the surface-adhered microbes. In one approach, X-ray surface-scattering techniques have been combined with X-ray absorption spectroscopy techniques to investigate selenium and lead speciation in biofilms (Templeton et al. 2001, 2003). In an alternative approach (Kemner et al. 2004), we used high-energy XRF microscopy to examine elemental compositions of single hydrated bacterial cells in both surface-adhered and planktonic states. We have also combined XAFS techniques with XRF microscopy approaches (XRF microspectroscopy) to determine the redox state and location of chromium relative to single surface-adhered and planktonic cells.

Results from XRF microscopy imaging experiments, depicted in FIGURE 4, demonstrate that the spatial distributions of many elemental macronutrients required for cell growth (phosphorus, sulfur, chlorine, potassium, and calcium) can enable imaging of single bacterial cells with resolution of approximately 100 nm (Kemner et al. 2004). We also observed changes in cell morphology and elemental concentrations in single cells attached to a solid substrate. Upon exposure to elevated concentrations of Cr^{6+}, we observed interactions between the cell surface and the chromium for the planktonic cells, but no interactions for the surface-adhered cells. In addition, XRF-microscopy analyses of macronutrient concentrations in the cells were consistent with death of the planktonic cells but not the surface-adhered cells. Finally, to determine the spatial distribution of the redox states of chromium in these systems at a number of locations relative to the cell, we measured XANES at the chromium K-edge with the X-ray microbeam (spatial resolution of approximately 100 nm) (Koningsberger and Prins 1988). Comparison of the chromium XANES spectra from these samples with spectra for standards indicated reduced chromium complexed to a phosphoryl functional group in contact with the cell surface of the planktonic cells but not in contact with the cell surface of the surface-adhered cells. Copious amounts of extracellular polysaccharides (EPS) and reducing end sugars residing within EPS are often observed to be produced in conjunction with the adhesion of cells to surfaces. Our results suggest that the EPS and reducing sugars impart to surface-adhered cells a tolerance to Cr^{6+} and the ability to reduce and immobilize the metal outside the cell. Similarly, the absence of these products around the planktonic cells renders them susceptible to the strong oxidizing power of the Cr^{6+}. The result is ruptured cell membranes and cell death upon exposure to Cr^{6+}.

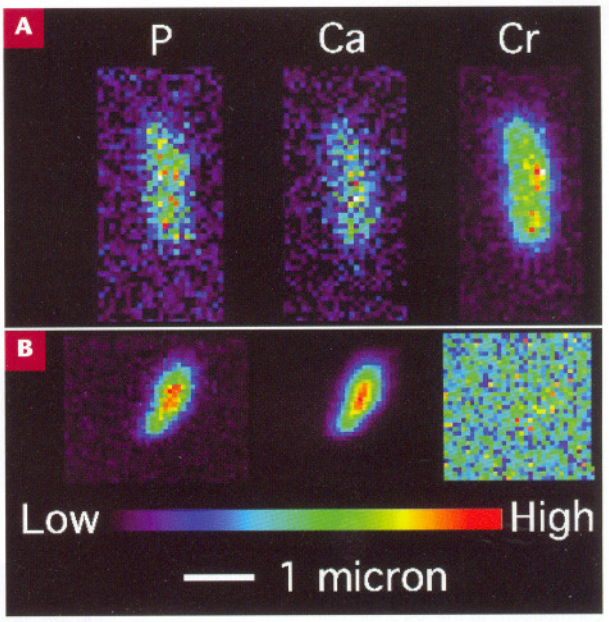

FIGURE 4 False-color micro-XRF maps of the qualitative distribution and concentration gradients of phosphorus, calcium, and chromium in and around (**A**) planktonic and (**B**) surface-adhered *Pseudomonas fluorescens* microbes harvested after exposure to potassium dichromate (Cr^{6+}) solution (1000 ppm) for 6 h.

This was one of the first studies of its kind at this spatial resolution; clearly, the use of XRF microscopy and microspectroscopy holds great promise for investigating a cell's metabolic state. Future studies of this type should also provide insight into biomineralization processes (i.e. the formation of minerals within and near microbes), another forefront research topic in the study of mineral–microbe interactions.

SUMMARY

The importance of microbes and microbe–mineral interactions in metal transformations is increasingly recognized. With newly developed synchrotron-based X-ray sources, our ability to characterize the chemical states of dilute elements in a variety of systems is enhanced. The studies presented here provide new insights concerning (1) chemical interactions between cell surfaces and metals, (2) the formation and chemistry of nanoparticles, and (3) changes in a cell's resistance to heavy metals upon attachment to surfaces. None of these new insights were possible solely with classical macroscale techniques. Synchrotron capabilities hold great promise for more sophisticated studies and increased understanding of metal transformations at mineral–microbe interfaces, as well as progression toward investigation of mineral–microbe interfaces in natural samples.

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